



ACADEMIC  
PRESS

Methods 26 (2002) 199–213

METHODS

www.academicpress.com

## Analysis of gene function in somatic mammalian cells using small interfering RNAs

Sayda M. Elbashir,<sup>a,1</sup> Jens Harborth,<sup>b,1</sup> Klaus Weber,<sup>b,\*</sup> and Thomas Tuschl<sup>a,\*</sup>

<sup>a</sup> Department of Cellular Biochemistry, Max-Planck-Institute for Biophysical Chemistry, Am Fassberg 11, D-37077 Göttingen, Germany

<sup>b</sup> Department of Biochemistry and Cell Biology, Max-Planck-Institute for Biophysical Chemistry, Am Fassberg 11, D-37077 Göttingen, Germany

Accepted 29 January 2002

### Abstract

RNA interference (RNAi) is a highly conserved gene silencing mechanism that uses double-stranded RNA (dsRNA) as a signal to trigger the degradation of homologous mRNA. The mediators of sequence-specific mRNA degradation are 21- to 23-nt small interfering RNAs (siRNAs) generated by ribonuclease III cleavage from longer dsRNAs. Twenty-one-nucleotide siRNA duplexes trigger specific gene silencing in mammalian somatic cells without activation of the unspecific interferon response. Here we provide a collection of protocols for siRNA-mediated knockdown of mammalian gene expression. Because of the robustness of the siRNA knockdown technology, genome-wide analysis of human gene function in cultured cells has now become possible. © 2002 Elsevier Science (USA). All rights reserved.

**Keywords:** RNA interference; Small interfering RNA; Posttranscriptional gene silencing; Knockdown; Double-stranded RNA

### 1. Introduction

Mammalian gene function has been determined traditionally by methods such as disruption of murine genes, the introduction of transgenes, the molecular characterization of human hereditary diseases, and targeting of genes by antisense or ribozyme techniques. In addition, microinjection of specific antibodies into cultured cells or binding of antibodies to cell surface-exposed receptors may provide information on the function of the targeted protein. A new alternative to these reverse genetic approaches has now become available with the discovery of small interfering RNAs, which are able to trigger RNA interference in mammalian somatic cells [1,2].

RNA interference (RNAi) is a sequence-specific posttranscriptional gene silencing mechanism, which is triggered by double-stranded RNA and causes degradation of mRNAs homologous in sequence to the

dsRNA [3,4]. Although RNAi has been observed in a wide range of eukaryotes, including plants, protists, filamentous fungi, and invertebrate and vertebrate animals [5–10], it has only recently become possible to silence human genes in cultured somatic cells [1]. The detection of RNAi in somatic mammalian cells has been hampered by the presence of a number of dsRNA-triggered pathways that mediate nonspecific suppression of gene expression [11–14]. dsRNA is a potent inducer of type I interferon (IFN) synthesis and is the activator of two classes of IFN-induced enzymes: PKR, the dsRNA-dependent protein kinase, and 2',5'-oligoadenylate synthetases, whose products activate the latent ribonuclease RNase L. These nonspecific responses to dsRNA are not triggered by dsRNA shorter than 30 bp including siRNA duplexes that resemble in length and structure the natural processing products from long dsRNAs [1,2,15]. The most predominant processing products are duplexes of 21- and 22-nt RNAs with symmetric 2-nt 3' overhangs, which are also the most efficient mediators of mRNA degradation [16].

One of the enzymes involved in processing long dsRNAs to siRNA duplexes is the RNase III enzyme Dicer, which was characterized in extracts prepared

\* Corresponding authors. Fax: +49-551-201-1197.

E-mail addresses: office.weber@mpibpc.gwdg.de (K. Weber),  
tuschl@mpibpc.gwdg.de (T. Tuschl).

<sup>1</sup> Authors contributed equally to this review.